

Application No. 09/121,798
Amendment Dated June 11, 2004
Reply to Office Action of February 11, 2004

Please replace paragraph [0040] with the following amended paragraph:

[0040] A chromatography column is packed with an anion exchange chromatography resin. The optimal capacity of the column is determined empirically based on the resin used and the size of the nucleic acid to be purified. The column is packed under low pressure, typically less than about [[7]] 0.7 bar. The pressure will depend on the resin used, and will usually be according to the manufacturer's specifications. Normal column operating pressure may be lower where the resin pore size is smaller, to limit trapping of the nucleic acid in the resin pores. Thus, for resins without pores, column operating pressure may be increased. The column is packed at about twice the anticipated flow rate in accordance with conventional techniques.

0065 *4/2 5/3/2010*
Please replace paragraph [0064] with the following amended paragraph:

[0064] The cell pellets were spread into thin sheets and frozen at -80° C. until used for further plasmid purification. 3.2 Kg of the cell pellet was resuspended in 16L Solution I (25 mM Tris-HCl, pH 8, 10 mM EDTA, 50 mM dextrose) at room temperature with stirring at 150 rpm for 1 h. RNase digestion was achieved by the addition of RNase (305 mg RNase/Kg cell paste) and incubating the solution on ice for 2 hrs. Cells were lysed by the addition of the cells to 32L Solution II (0.2N NaOH/1%SDS) in an ice bath. The solution is stirred using a Bow-Tie Stirrer (Cole Parmer, Vernon Hills, Ill.) for 25 min. This solution was then neutralized and cell debris and chromosomal DNA were precipitated by the addition of 16L ice-cold Solution III (3M potassium, [[SM]] 5M acetate, pH 5.5). The solution was mixed with a Bow-Tie Stirrer on ice for 25 min.

0084 *4/2 5/3/2010*
Please replace paragraph [0083] with the following amended paragraph:

[0083] Approximately 40 L of fermentation broth yields about 2.2 kg of cell paste. After re-suspension of the cell paste, lysis and precipitation, approximately 40 liters of solution were ready for clarification by centrifugation. Centrifuging in a non-continuous centrifuge (Sorvall RC3b) at 7500 x g for 25 minutes removed the solids and yielded a clarified product. Tris base (solid) was added to adjust the pH of the clarified product to 8.5 (a final concentration of 0.67 M). After Tris base addition, the conductivity decreased from 53 mS/cm to 50 mS/cm. The neutralized lysate was filtered in series with a nominal 0.2 μ m glass filter (Sartopure GF) and an absolute [[0.2 m]] 0.2 μ m nylon filter (Pall Ultipor N66) (5 ft² each) to reduce bacterial load and endotoxin levels.

Application No. 09/121,798
Amendment Dated June 11, 2004
Reply to Office Action of February 11, 2004

0086

4/25/2010

Please replace paragraph [0085] with the following amended paragraph:

[0085] The pooled product contained 1787 mg DNA, endotoxin level of 16 EU/mg, and 1.6% genomic DNA. The product was filtered again through [[0.2 m]] 0.2 μ m nominal glass and [[0.2 m]] 0.2 μ m absolute nylon filters described above. After filtering the product contained endotoxin level of 1 EU/mg and 0.18% genomic DNA, with 94% yield. The filtered product was diafiltered and subjected to a final [[0.2 m]] 0.2 μ m sterilization filter as described in Example 1. The final product was 5.6 mg/ml, with 0.4 EU/mg and less than 0.2% genomic DNA.

Please replace paragraph [0064] with the following amended paragraph:

[0064] The cell pellets were spread into thin sheets and frozen at -80° C. until used for further plasmid purification. 3.2 Kg of the cell pellet was resuspended in 16L Solution I (25 mM Tris-HCl, pH 8, 10 mM EDTA, 50 mM dextrose) at room temperature with stirring at 150 rpm for 1 h. RNase digestion was achieved by the addition of RNase (305 mg RNase/Kg cell paste) and incubating the solution on ice for 2 hrs. Cells were lysed by the addition of the cells to 32L Solution II (0.2N NaOH/1%SDS) in an ice bath. The solution is stirred using a Bow-Tie Stirrer (Cole Parmer, Vernon Hills, Ill.) for 25 min. This solution was then neutralized and cell debris and chromosomal DNA were precipitated by the addition of 16L ice-cold Solution III (3M potassium, [[SM]] 5M acetate, pH 5.5). The solution was mixed with a Bow-Tie Stirrer on ice for 25 min.

0064 5/3/2010

Please replace paragraph [0065] with the following amended paragraph:

[0065] The precipitated material was removed from the neutralized cell lysis solution by centrifugation. The solution was aliquoted into [[IL]] 1L centrifuge bottles and centrifuged at 5300 rpm for 20 min at 2° C. The supernatants were then decanted through two layers Miracloth (CalBiochem, La Jolla, Calif.) arranged at 90° to each other, into a container at room temperature. The decanted supernatants were then filtered through 1.2 and [[0.21]] 0.2 μ m filters arranged in series. As an alternative to centrifugation at this stage, precipitated material may be removed by filtration through a diatomaceous earth material such as Celite® HYFLO SUPER CEL® (Celite Corp., Lompoc, Calif.). See U.S. Pat. No. 5,576,196.

0065 5/3/2010

Please replace paragraph [0069] with the following amended paragraph:

[0069] Spectrophotometric analysis was performed at wavelengths of 250, 260, and 280 [[m]] nm. Typical ratios for purified DNA are $OD_{260}/OD_{280} > 1.9$. A total of 2.307 g of plasmid DNA was isolated and purified in the above procedure, having OD_{260}/OD_{250} of 1.1047 and OD_{260}/OD_{280} of 1.9290.

Please replace paragraph [0083] with the following amended paragraph:

[0083] Approximately 40 L of fermentation broth yields about 2.2 kg of cell paste. After re-suspension of the cell paste, lysis and precipitation, approximately 40 liters of solution were ready for clarification by centrifugation. Centrifuging in a non-continuous centrifuge (Sorvall RC3b) at 7500 x g for 25 minutes removed the solids and yielded a clarified product. Tris base (solid) was added to adjust the pH of the clarified product to 8.5 (a final concentration of 0.67 M). After Tris base addition, the conductivity decreased from 53 mS/cm to 50 mS/cm. The neutralized lysate was filtered in series with a nominal 0.2 μ m glass filter (Sartopure GF) and an absolute [[0.2 m]] 0.2 μ nylon filter (Pall Ultipor N₆₆) (5 ft² each) to reduce bacterial load and endotoxin levels.